1: Invest Ophthalmol Vis Sci. 1998 Jul;39(8):1346-51.

Links

Reoxygenation injury in a cultured corneal epithelial cell line protected by the uptake of lactoferrin.

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PURPOSE: To investigate whether reoxygenation after extended hypoxia causes cellular damage in cultured corneal epithelial cells and to demonstrate the protective effects of lactoferrin. METHODS: Immortalized human corneal epithelial cells (T-HCECs) were cultured to confluence in 96-well culture plates, subjected to stringent hypoxia (1% O2, 5% CO2, 94% N2 at 37 degrees C) for 24 hours, and returned to normoxic conditions (5% CO2, 95% air at 37 degrees C). Cell viability was observed by 1 microM propidium iodide staining 0, 2, 4, and 6 hours after reoxygenation. Inhibition studies were performed after 2 hours' reoxygenation, using 2 mM iron chelator desferrioxamine and 0.2 mg/ml lactoferrin. Confocal immunocytochemistry for human lactoferrin and western blot analysis for lactoferrin-induced ferritin were performed in cultured T-HCECs to demonstrate the internalization of lactoferrin after application. RESULTS: After 2 hours, reoxygenation of T-HCECs after hypoxia produced an increase in cell death that was significantly greater than that observed in normoxic control cells or in cells subjected to hypoxia for the same time span without reoxygenation. The addition of desferrioxamine and lactoferrin at the time of reoxygenation significantly attenuated cellular damage. Confocal immunocytochemistry revealed that lactoferrin is taken into the cytoplasm of T-HCECs as early as 30 minutes after application. This was also demonstrated in western blot analysis by the upregulation of intracellular ferritin at 18 hours by the addition of iron-bound lactoferrin but not by iron-free lactoferrin. CONCLUSION: Reoxygenation is responsible for increased cellular damage after extensive hypoxia, which is attenuated by chelators of free iron in the cytosol, including the major tear protein lactoferrin.

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